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GROUP 120

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Horst Meyer, et al.
Serial No. : 27,540
Filed : April 5, 1979
For : CEREBRAL THERAPEUTIC AGENT AND ITS USE
Art Unit : 125
Examiner : Friedman

Honorable Commissioner of Patents
and Trademarks
Washington, D. C. 20231

SIR:

D E C L A R A T I O N

I, Dr. S. Kazda, hereby declare that all statements made herein of my own knowledge are true and that statements made on information and belief are believed to be true; and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above identified patent application or any patent issuing thereon:

1. I am a citizen of the Federal Republic of Germany, residing at Pahlkestr. 55 N, 5600 Wuppertal 1.
2. I graduated from the Medical School of Charkow in 1957 and was awarded the degree of a doctorate of medicine (1957) and of a "candidatus scientiarum" (Ph. D., 1961) from the Charles University of Prague.
3. On May 1, 1971 I joined BAYER AG as a head of a pharmacological laboratory and 1976 as a head of the Department of cardiovascular pharmacology in their Institute of Pharmacology.
On April 30, 1976 I obtained from the Medical Council Nordrhein the qualification "Facharzt für Pharmakologie" (medical specialist on pharmacology).
4. I have had carried out under my direction passive avoidance tests of (A) compounds used in the invention of the above-identified patent application and (B) compounds used in the prior art.
5. Said passive avoidance tests were carried out

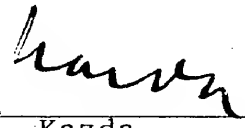
using essentially the procedure of Sara et al, Psychopharmacol. 25, 32-40 (1972), more specifically according to the attached Exhibit A.

6. The results of said passive avoidance tests are tabulated in the enclosed Exhibit B in which Compound Bay e 9736 is the compound used in the instant invention; Compounds Bay h 2414 and Bay h 3662 are compounds of reference U.S. Patent 2,799,934 and Compound YC-93 is a compound of reference U.S. Patent 3,985,758.
7. The results clearly show that where the use in the instant invention of Compound Bay e 9736 is effective for the intended use with an ED₅₀ of 0.16 mg/kg p.o., typical reference compounds including an adjacent homolog have an undetermined ED₅₀ which is more than 1 mg/kg p.o.

Further declarant saith not.

Date:

April 17, 1980.



Dr. S. Kazda

Test procedure for passive-avoidance-test

Adult male lean "Zucker" rats weighing 170 - 230 g were used in all experiments. Each experimental group included 15 rats.

The passive avoidance test chamber consisted of a grid floor, a large light compartment (29 by 21 by 21 cm), and a smaller dark compartment (10 by 21 by 21 cm) which were connected by a guillotine door (5 by 5 cm).

Each animal was placed in the light compartment and allowed to explore the whole training box for 3 min. Nearly all rats entered the dark compartment within a latency period of 20 - 40 sec. At the end of the exploration period (first test day) the animal was confined to the dark compartment where it received an electric foot shock of 1.6 mA for 20 sec. The rats were then removed from the test chamber and placed in air-tight cages gassed with 3,8 % O₂/96,2 % N₂ or room air, respectively. After onset of gasping in the hypoxia-treated animals the rats were placed in their home cages again.

24 h later (second test day) each animal was again placed into the light part of the training box and the latency to enter the dark compartment was measured. Maximal latency was defined 180 sec.

Drug treatment occurred 30 min before the first test.

For the calculation of protective effects of drug treatment three mean latency values were used:

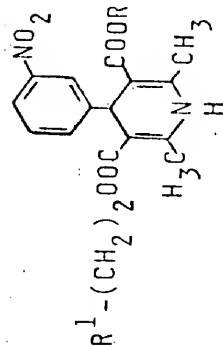
A: control, hypoxia

B: control, air

C: drugtreatment, hypoxia

$$\text{Protective effect (\%)} = \frac{C - B}{A - B} \cdot 100.$$

PROTECTION AGAINST HYPOXIAINDUCED AMNESIA



(Passive-avoidance-Test/Ratte)

S. J. Sara et al., Psychopharmacol.
25, 32-40 (1972)

Compound	R	R ¹	Dose mg/kg p.o.	% Protection	Results
Bay e 9736		OCH ₃	0,25 1,0	57 100	ED ₅₀ = 0,16 mg/kg p.o.
Bay h 2814		OC ₂ H ₅	0,3 1,0	21 22	ED ₅₀ not determined (» 1 mg/kg p.o.)
Bay h 3662		OCH ₃	0,3 1,0	30 30	
YC-93			0,3 1,0	29 21	